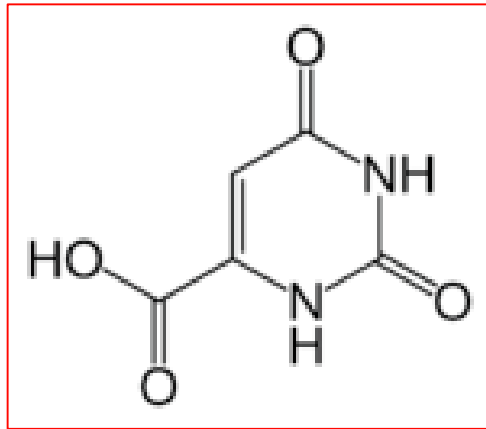


Quantitative Analysis of Orotic Acid by LC-ESI-MS/MS



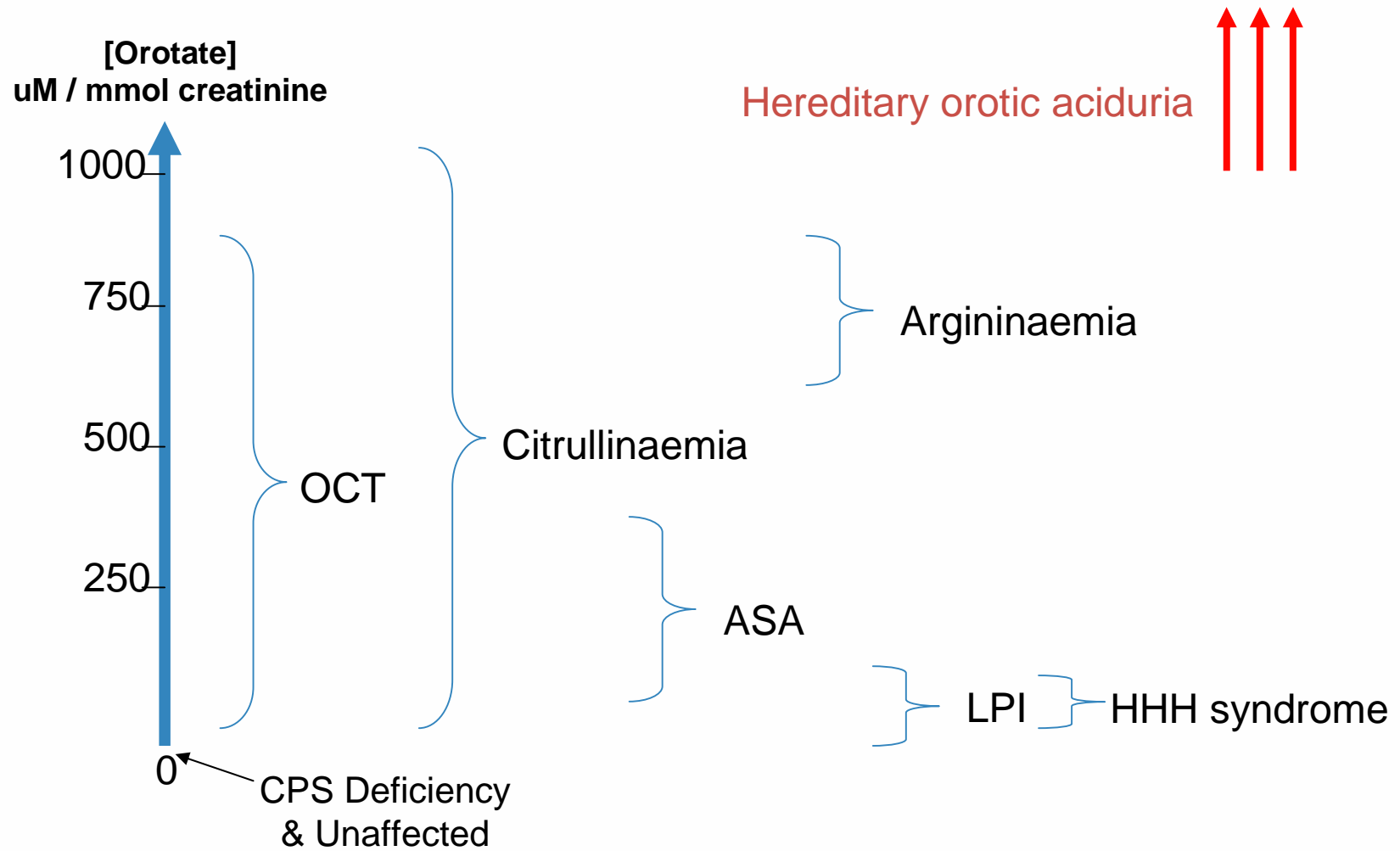
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Overview

- Increased orotate excretion a biomarker for several IEMs
- Quantitation of orotate useful since:
 - Can help with differential diagnosis
 - ✦ QAA not conclusive
 - ✦ Qualitative organic acid analysis misleading
- Stable isotope dilution LC-ESI-MS/MS method^[1]
 - Sample preparation rapid and simple
 - Good accuracy
 - Good precision
 - Capable of detecting elevated concentrations of clinical significance

Adapted from [1] Marca GI, Casetta B and Zammarchi E. Rapid determination of orotic acid in urine by a fast liquid chromatography/tandem mass spectrometric method. *Rapid Commun Mass Spectrom* 2003; 17:788-793.

Why Quantitate?



[2] Salerno C, Crifco C. Diagnostic value of urinary orotic acid levels: Applicable Separation Methods. J. Chrom B 781 (2002) 57-71

Choice of Instrumentation

HPLC

- Long procedure
 - Clean up
- Drug interference

GC-MS

- Long procedure
 - Clean-up/extraction
 - Derivatisation

LC-ESI-MS/MS

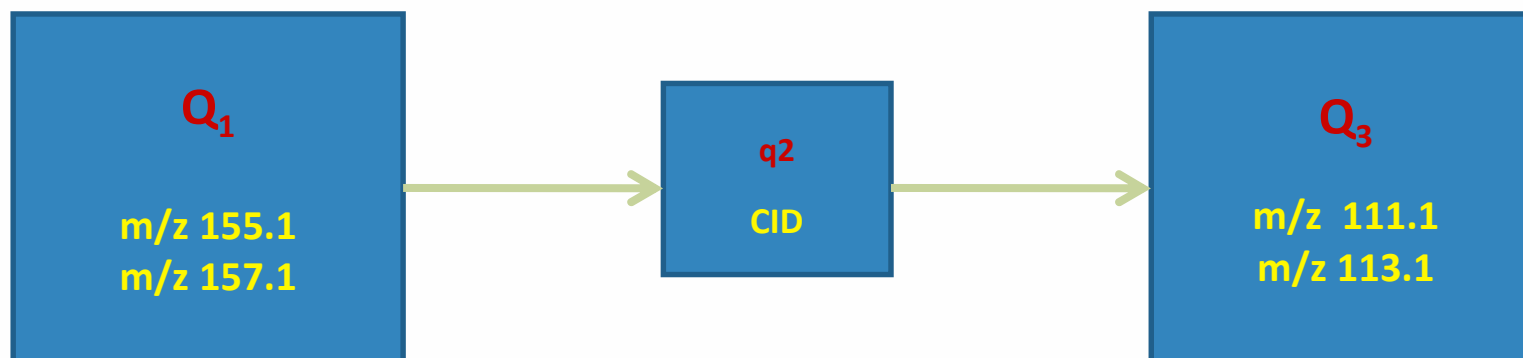
- Simple, rapid sample preparation:
 - 20ul sample + 150ul ¹⁵N₂-orotic acid (IS) (200μM)
 - ✦ Calibration standards 0-200μM
 - ✦ EQA samples used as IQC
- C18 reversed phase LC separation
 - 5μ x 4.6mm x 150mm Phenomenex Gemini
 - 60% acetonitrile with 2.5mM ammonium acetate

Multiple Reaction Monitoring (MRM)

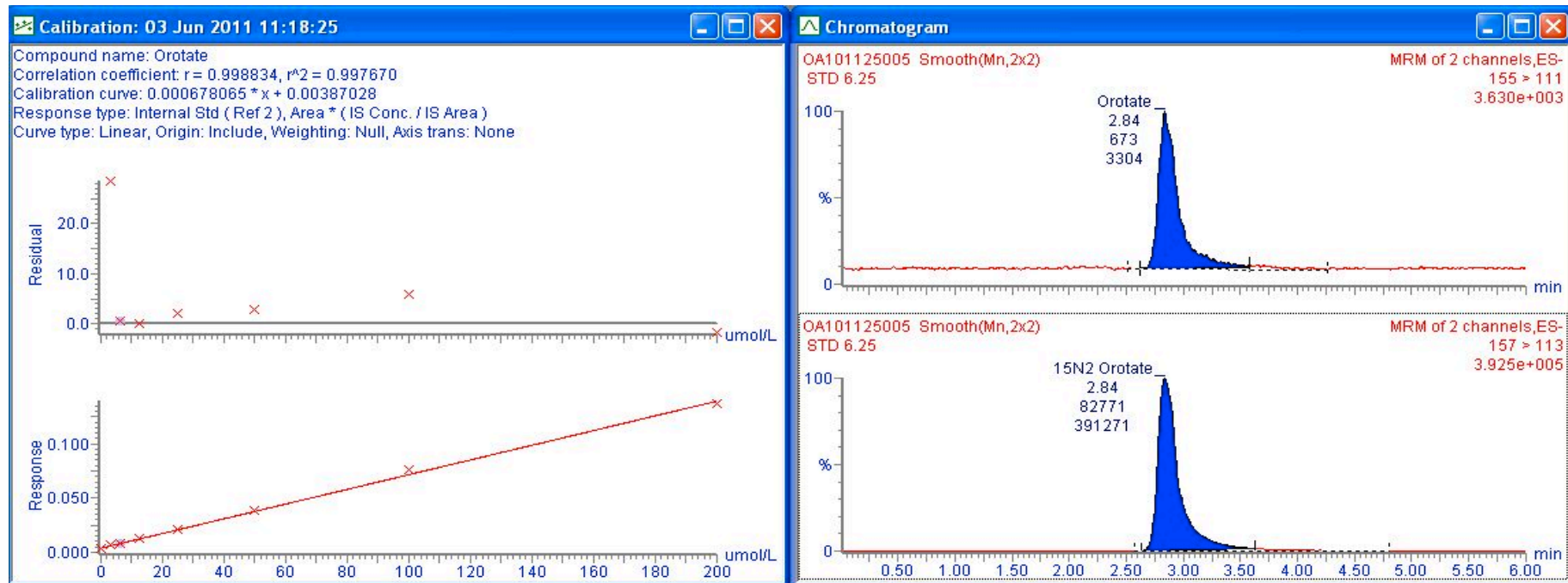
LC-ESI(-ve)-MS/MS in MRM mode

Orotic acid $[M-H]^-$ m/z 155.1 \rightarrow 111.1 $[M-H - CO_2]^-$

$^{15}N_2$ -Orotic acid $[M-H]^-$ m/z 157.1 \rightarrow 113.1 $[M-H - CO_2]^-$



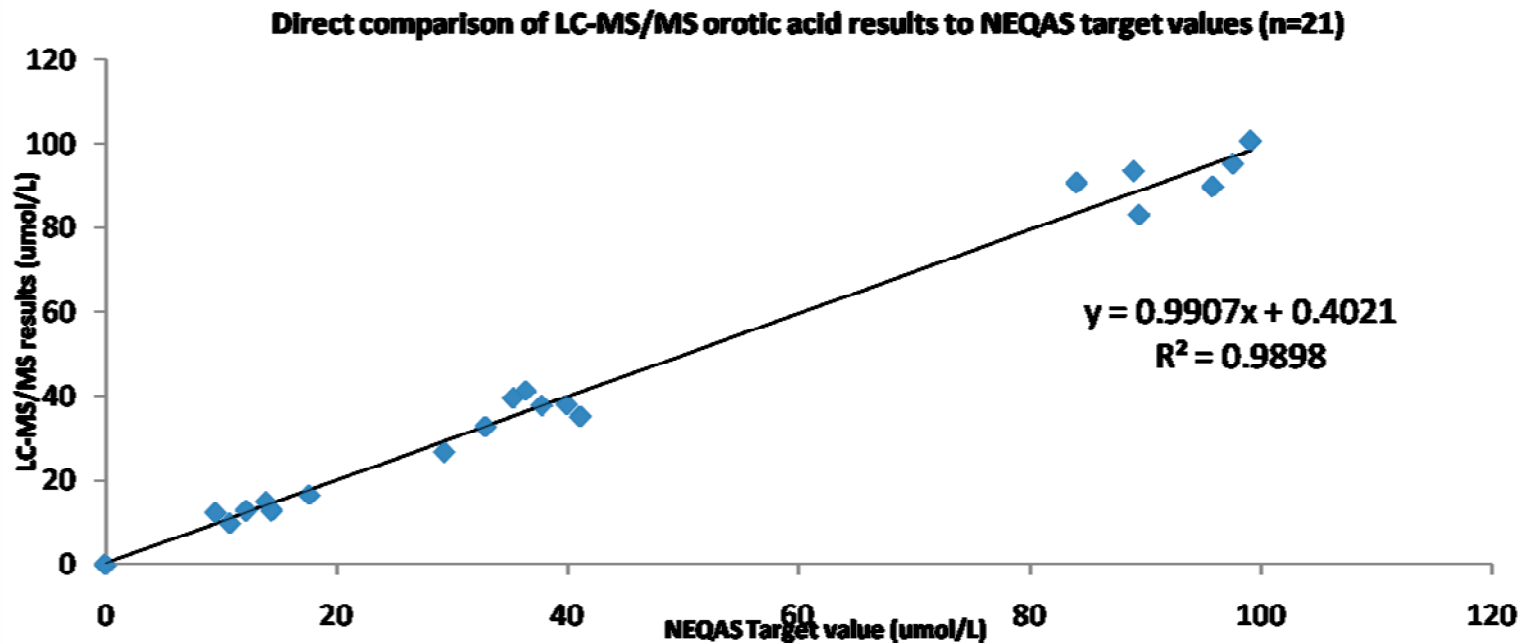
Linearity



- Linear to 200 μM
- $r^2 = 0.9977$

Accuracy

- 21 EQA samples analysed
- Direct comparison of results and target values
- $r^2 = 0.9898$



Precision

Inter-Assay

- EQA samples
 - $C_1 = 2.6 \mu\text{M}$
 - $C_2 = 39.9 \mu\text{M}$
 - $C_3 = 89.5 \mu\text{M}$
- $CV_1 = 29\%$ (n=7)
- $CV_2 = 8\%$ (n=11)
- $CV_3 = 7\%$ (n=11)

Intra-Assay

- 22 replicates of EQA material analysed
- $C = 36.5 \mu\text{M}$
- $CV = 6\%$

Limit of Detection

- LOD = 3.125 μM
- S/N = 42
- Fit for purpose – clinically significant increase detectable

